Efficacy of Tenofovir 1% Vaginal Gel in Reducing the Risk of HIV-1 and HSV-2 Infection

McConville, C., Boyd, P., & Major, I. (2014). Efficacy of Tenofovir 1% Vaginal Gel in Reducing the Risk of HIV-1 and HSV-2 Infection. *Clinical Medicine Insights: Women's Health*, *7*, 1-8. https://doi.org/10.4137/CMWH.S10353

Published in:
Clinical Medicine Insights: Women's Health

Document Version:
Publisher's PDF, also known as Version of record

Libertas Academica
FREEDOM TO RESEARCH

Open Access: Full open access to this and thousands of other papers at http://www.la-press.com.

Clinical Medicine Insights: Women's Health

Efficacy of Tenofovir 1% Vaginal Gel in Reducing the Risk of HIV-1 and HSV-2 Infection

Christopher McConville[1], Peter Boyd[2] and Ian Major[3]

[1]Department of Pharmacy, Faculty of Science and Engineering, University of Wolverhampton, Wolverhampton, UK. [2]School of Pharmacy, Medical Biology Centre, Queen's University of Belfast, Belfast, Northern Ireland, UK. [3]Materials Research Institute, Athlone Institute of Technology, Athlone, Westmeath, Ireland.

ABSTRACT: Human Immunodeficiency Virus (HIV) is a retrovirus that can result in rare opportunistic infections occurring in humans. The onset of these infections is known as Acquired Immune Deficiency Syndrome (AIDS). Sexual transmission is responsible for the majority of infections 1, resulting in transmission of HIV due to infected semen or vaginal and cervical secretions containing infected lymphocytes. HIV microbicides are formulations of chemical or biological agents that can be applied to the vagina or rectum with the intention of reducing the acquisition of HIV. Tenofovir is an NRTI that is phosphorylated by adenylate kinase to tenofovir diphosphate, which in turn competes with deoxyadeosine 5'-triphosphate for incorporation into newly synthesized HIV DNA. Once incorporated, tenofovir diphosphate results in chain termination, thus inhibiting viral replication. Tenofovir has been formulated into a range of vaginal formulations, such as rings, tablets gels and films. It has been shown to safe and effective in numerous animal models, while demonstrating safety and acceptability in numerous human trials. The most encouraging results came from the CAPRISA 004 clinical trial which demonstrated that a 1% Tenofovir vaginal gel reduced HIV infection by approximately 39%.

KEYWORDS: tenofovir, HIV, HSV, microbicide, vaginal gel

CITATION: McConville et al. Efficacy of Tenofovir 1% Vaginal Gel in Reducing the Risk of HIV-1 and HSV-2 Infection. *Clinical Medicine Insights: Women's Health* 2014:7 1–8 doi:10.4137/CMWH.S10353.

RECEIVED: October 16, 2013. **RESUBMITTED:** December 26, 2013. **ACCEPTED FOR PUBLICATION:** December 30, 2013.

ACADEMIC EDITOR: Goberdhan P. Dimri, Editor in Chief

TYPE: Review

FUNDING: Authors disclose no funding sources.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

CORRESPONDENCE: c.mcconville@wlv.ac.uk

Introduction

HIV and AIDS. Human immunodeficiency virus (HIV) is a retrovirus that can result in rare opportunistic infections occurring in humans. The onset of these infections is known as acquired immunodeficiency syndrome (AIDS). HIV is subdivided into HIV-1 and HIV-2. HIV-2 is largely confined to West African countries, and is extremely rare in Europe, Central or East Africa and North America. While HIV-2 is similar to HIV-1, it has a different sequence of nucleotides in its genome. The major modes of HIV transmission are sexual contact, exposure to infected blood, infected needles and mother-to-child. Sexual transmission is responsible for the majority of infections;[1] HIV is transmitted via infected semen or vaginal and cervical secretions containing infected lymphocytes.[2]

HIV destroys the human immune system by attacking the CD4+ T helper cells, a subgroup of lymphocytes, which are a type of white blood cell that is part of the adaptive immune system.[3,4] This leaves the body susceptible to opportunistic infections, which leads to the onset of AIDS. HIV consists of a genome containing two identical single strands of RNA along with two molecules of reverse transcriptase that copies RNA into DNA. Two proteins, known as *p7* and *p9* are also associated with the genome, which is then surrounded by *p17* proteins on the outer core and *p24* on the inner core. Surrounding these core proteins is an envelope that contains two HIV-specific glycoproteins, *gp41* and *gp120* (see Fig. 1).[5]

All retroviruses have three common genes, *gag*, *pol* and *env*, which code for the main polyproteins of the virus. These polyproteins, when cleaved by viral protease, result in the production

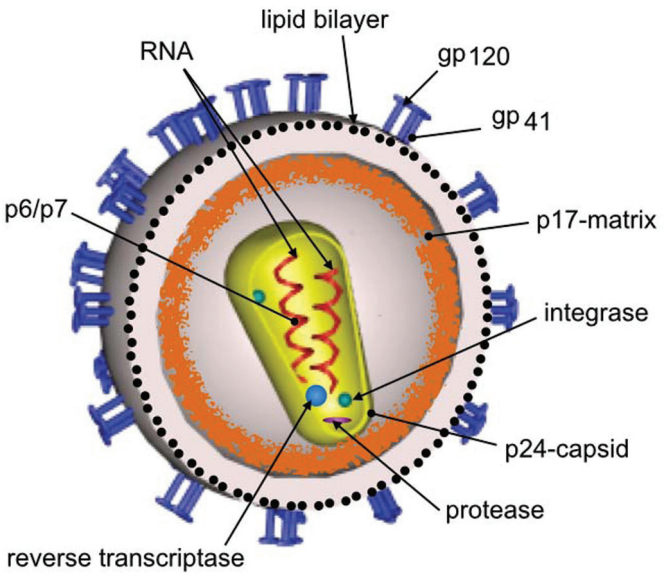

Figure 1. Structure of HIV.

of the core proteins (*p7*, *p9*, *p17* and *p24*), the replication enzymes (reverse transcriptase, protease and intergrase) and finally the envelope proteins (*gp41* and *gp120*).[6] HIV contains six unique genes that code for proteins required to regulate the expression of the HIV genome. Two of these genes are *tat* and *rev,* which code for a trans-activator protein and a regulator of mRNA transcription, respectively. The *tat* protein binds to an RNA sequence on the genome known as TAR (trans-activation response element), which results in an increase in the number of RNA transcripts formed.[7] The HIV genome also includes *vif*, *upr*, *nef* and *upu* genes, which help in the regulation of transcription.

HIV replication. The HIV replication cycle begins with attachment of the virus to CD4 receptors on certain cells of the immune system (T helper cells, lymphocytes and macrophages) and glial cells on the brain (see Fig. 2). Viral attachment occurs via the *gp120* envelope proteins (there is estimated to be 220 on each virion).[8] Upon attachment *gp120* interacts with another protein on the host cell surface, CD26 (not shown in Fig. 2). This interaction results in the exposure of a site on the *gp41* viral envelope protein that fuses the viral envelope with the host cell cytoplasmic membrane, resulting in the entry of the virus into the host cell.[9] The viral coat is removed and the single-stranded RNA genome is reverse-transcribed to double-stranded cDNA by the enzyme reverse transcriptase.

This proviral DNA is transported into the host cell nucleus and integrated with the host genome at specific sites along the chromosome, by the viral enzyme integrase.[10] This integrated viral genome is known as a provirus and is transcribed and translated into new viral proteins.[5] If the proviral DNA is activated it can produce new strands of RNA. This RNA either becomes messenger RNA, and is used for the production of viral proteins, or becomes encased within the viral core to become the new virus.[6]

The *gag* gene is transcribed and translated into a polyprotein called *p53*, which is then cleaved into the core proteins *p7*, *p9*, *p17* and *p24* by the HIV-coded protease. The *pol* gene is also transcribed, translated and proteolytically cleaved into reverse transcriptase, protease and intergrase polypeptides.[11] The last gene to be transcribed and translated into the polyprotein *gp160* is the *env* gene. Then, *gp160* is cleaved into the envelope glycoproteins *gp120* and *gp41,* which are incorporated into the host's cytoplasmic membrane. The viral particles are then assembled and released slowly from the infected host cell by a process known as 'budding' (see Fig. 2).[12]

HIV Microbicides

HIV microbicides are formulations of chemical or biological agents that can be applied to the vagina or rectum with the intention of reducing the likelihood of acquisition of HIV. An effective microbicide product has the potential to reduce the global HIV infection rate.[13–15] The ideal vaginal HIV microbicide must have activity against cell-free and cell-associated HIV. It must not cause damage to the tissue or flora of the vagina. It must be retained in the vagina, act locally and retain its activity in the presence of semen and across a broad pH range.[16]

There are various mechanisms by which vaginal HIV microbicides may prevent HIV infection (see Fig. 3): 1) by destroying the virus as soon as it enters the vagina,[17,18] 2) maintenance of the vaginal flora, which provides a protective vaginal pH,[19,20] 3) prevention of HIV binding to CD4 receptors,[21,22] 4) by preventing the HIV replication process,[23,24] 5) by providing a physical barrier that prevents HIV from entering the vaginal mucosa,[25] and 6) by prevention of sexually transmitted infection (STIs), which may increase the possibility of HIV infection.[26]

Reverse transcriptase inhibiting HIV microbicides. Reverse transcriptase inhibitors (RTIs), which inhibit the viral encoded enzyme reverse transcriptase responsible for the conversion of single strand viral RNA into double-stranded DNA, are being evaluated as HIV microbicides. Both nucleotide and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs) are under evaluation. NRTIs inhibit the process of reverse transcriptase by insertion into the propagating viral DNA, thereby inhibiting further synthesis of DNA. NNRTIs inhibit reverse transcriptase by binding directly to the reverse transcriptase enzyme and inhibiting the conversion of viral RNA into viral DNA.[23,24]

Tenofovir. Tenofovir (PMPA) is an NRTI that is phosphorylated by adenylate kinase to tenofovir diphosphate, which in turn competes with deoxyadeosine 5'-triphosphate for incorporation into newly synthesized DNA. Once incorporated, tenofovir diphosphate results in chain termination, thus inhibiting viral replication. Tenofovir is currently used in antiretroviral therapy for the treatment of HIV and is marketed under the brand name Truvada®, which is a once daily tablet containing 300 mg of tenofovir and 200 mg of emtricitabine. Truvada® has been approved by the Food and Drug

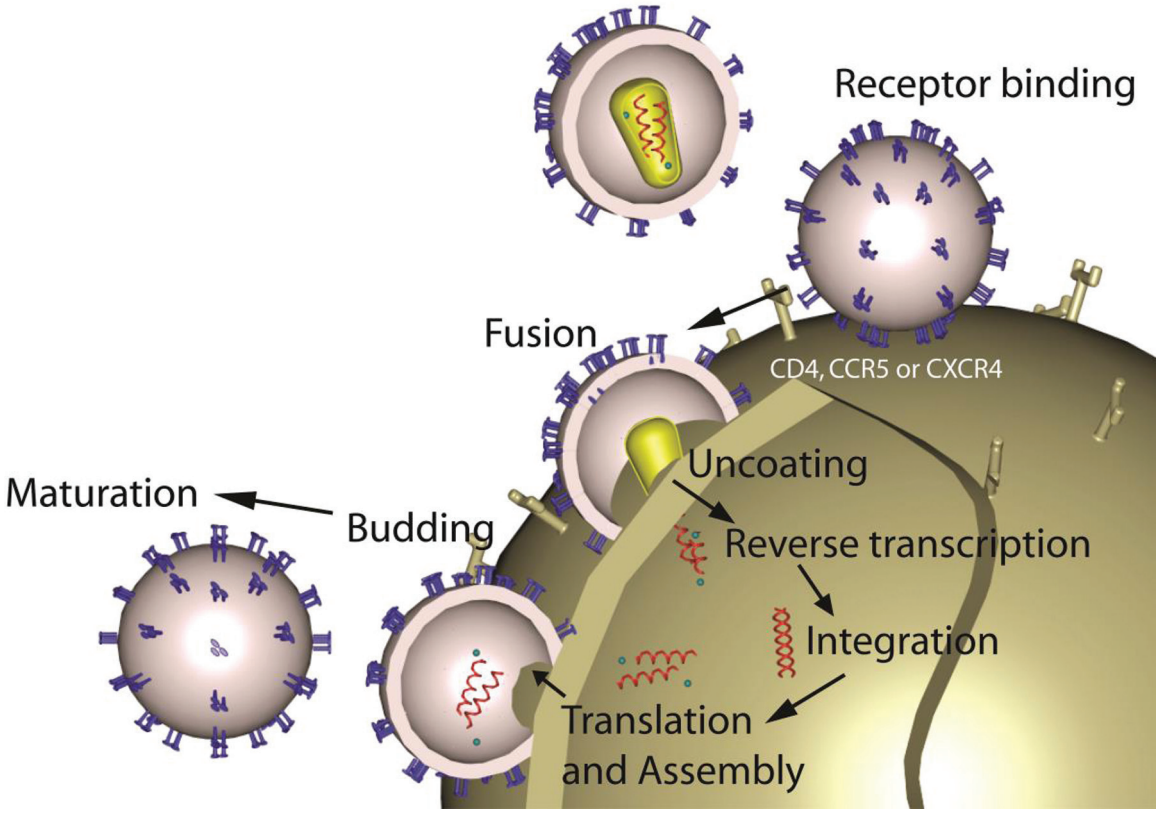

Figure 2. HIV replication cycle.

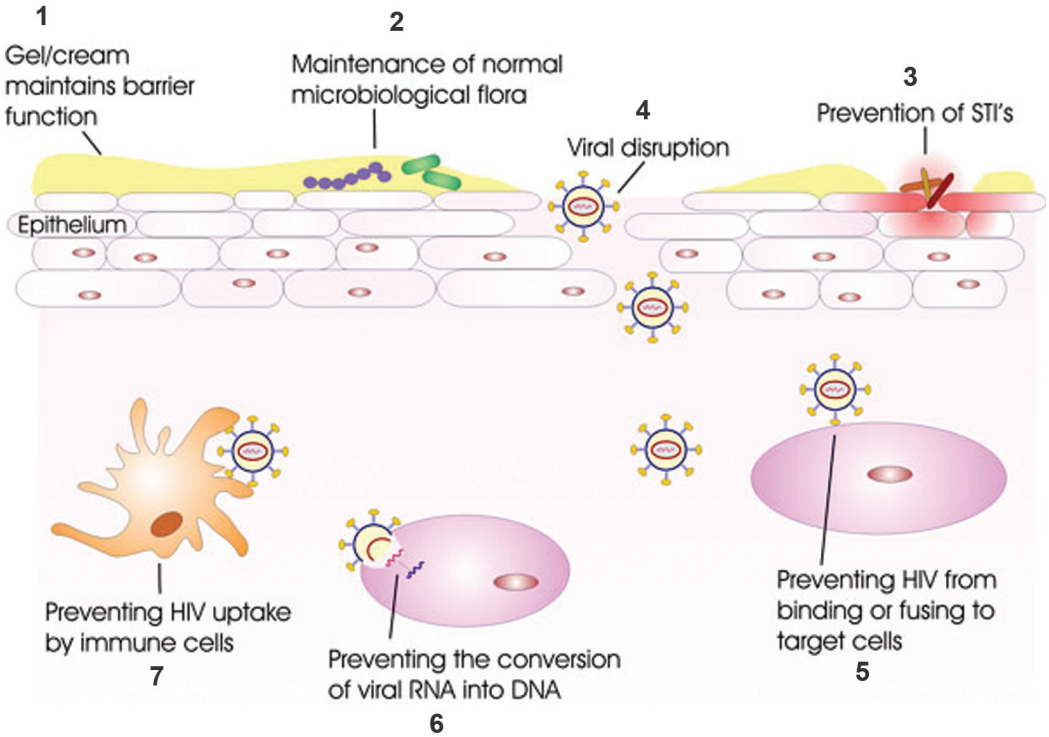

Figure 3. Potential mechanisms of HIV prevention by a microbicide formulation: (**1**) provision of a physical barrier that prevents HIV from entering the vaginal mucosa,[25] (**2**) maintenance of the vaginal flora, which provides a protective vaginal pH,[19,20] (**3**) prevention of sexually transmitted infections (STI's) which may increase the possibility of HIV infection,[26] (**4**) by destroying the virus as soon as it enters the vagina,[17,18] (**5**) prevention of HIV binding to CD4 receptors,[21,22] (**6**) preventing the HIV replication process[23,24] ultimately leading to the prevention of HIV uptake by the immune cells (**7**) (www.empro.org.uk).

Administration (FDA) for use as a pre-exposure prophylaxis strategy against HIV infection. Tenofovir is also marked as Atripla® (or Viraday®), which is a once daily combination tablet containing emtricitabine, tenofovir and efavirenz designed to increase compliance of antiretroviral therapy by reducing the pill-burden of HIV-positive patients. It has been demonstrated that there is less chance of HIV developing resistance to tenofovir compared to other reverse transcriptase inhibitors.[24] Tenofovir's efficacy, long half-life and safety profile make it an ideal candidate for use as an HIV microbicide strategy,[27] while a study performed in SIV-positive macaques concluded that when they were treated systemically with either 30 mg/Kg or 75 mg/Kg of PMPA the viral load was significantly reduced. However, the viral load increased when the treatment was stopped.[28] Tenofovir's potential for use as an HIV microbicide was further corroborated by successful invitro and invivo assessment of a 1% tenofovir gel,[27] while two macaque studies of tenofovir gels administered vaginally showed 100% and 80% protection.[29] Tenofovir's efficacy against viral challenge in animal models has been established using either pre- or post-exposure prophylaxis administration.[30–32]

Tenofovir has also been shown to be effective against the herpes simplex virus-2 (HSV-2), with studies demonstrating that a tenofovir vaginal gel not only reduces HIV infection but surprisingly also suppresses HSV-2 infection.[33] However, it has been demonstrated that administering tenofovir orally has no impact on HSV-2 infection.[34] Andrei et al demonstrated that tenofovir inhibits the replication of HSV in a range of human clinical isolates and decreases HSV replication in human lymphoid and cervicovaginal tissues ex vivo, while delaying HSV-induced lesions and death in topically treated HSV-infected mice. They concluded that tenofovir inhibits HSV-2 DNA-polymerase, but in order to achieve effective drug concentrations it must be administered topically rather than orally.[35]

Clinical Studies and Efficacy

Tenofovir has been comprehensively studied for safety, acceptability and efficacy. Primarily these studies have focused on the drug in an oral dosage form as part of antiretroviral (ARV) therapy and typically in combination with other antiretrovirals. A three-year trial evaluating the safety and efficacy of tenofovir vs. stavudine when taken in combination with lamivudine and efavirenz showed both compounds to be highly effective in ARV-naive patients, though tenofovir was associated with less toxicity than stavudine.[36] A study examining a three-drug regimen of tenofovir, abacavir and lamivudine in HIV-infected, ARV-naive subjects showed an unacceptably high virologic non-response, leading to the authors' conclusion that combination therapies should not be given based on presumed efficacy of the individual drugs.[37] In the CASTLE study, fixed dose tenofovir-emtricitabine was shown to be effective in combination with either atazanavir-ritonavir once daily or lopinavir-ritonavir twice daily.[38] The STEAL study compared safety and efficacy of once-daily, fixed dose combinations tenofovir-emtricitabine vs. abacavir-lamivudine, finding that both combinations had similar virological efficacy but abacavir-lamivudine was associated with more serious non-AIDS events, such as cardiovascular events.[39]

A phase 3 trial measured the efficacy of tenofovir-emtricitabine in HIV-1 infected, treatment-naive adults in combinational therapy with either efavirenz or a newer NNRTI, rilpivirine.[40] The study showed that the combination with rilpivirine had non-inferior efficacy compared to the combination with efavirenz, with higher virological failure but a more favourable safety and tolerability profile. A single dose tablet combining the integrase inhibitor elvitegravir co-formulated with cobicistat, emtricitabine and tenofovir has been trialled,[41] showing non-inferiority compared to the widely used combination efavirenz-emtricitabine-tenofovir. The new tablet offers the potential for a complete regimen in a single daily dose for initial treatment of HIV-1 infected patients. In protection against mother-to-child transmission, tenofovir-emtricitabine was trialled in combination with intrapartum and neonatal single-dose nevirapine and was shown to reduce viral resistance to NNRTIs at 2 and 6 weeks after ingestion.[42] A pharmacokinetic study involving oral tenofovir in combination with atazanavir-ritonavir in heavily pre-treated HIV infected patients suggested the existence of significant interaction between atazanavir-ritonavir and tenofovir.[43]

The use of these same tenofovir-based oral dosage forms is now being examined in a number of trials to measure the effectiveness of such combinations as a prevention strategy in HIV-uninfected persons to reduce the transmission of HIV. The results from these trials have been complex and contrasting. The FEM-PrEP (pre-exposure prophylaxis) trial studying African women was discontinued early because of a lack of protection.[44] Contrastingly, the TDF2 study found an efficacy rate of about 62% for HIV prevention in both African men and women.[45] The Partners PrEP study examined the use of daily oral tenofovir or tenofovir-emtricitabine in high-risk populations of sexually active women, men and HIV-discordant couples with an efficacy rate of approximately 75%.[46]

A phase 1 clinical trial of 0.3% and 1% tenofovir vaginal gels in sexually active and sexually inactive HIV-negative and HIV-positive women found the gels to be safe, acceptable and well tolerated for a two-week twice daily course.[47] A PK cross-over study comparing tenofovir vaginal gel and oral tablets found that gel dosing achieved lower serum concentrations but much higher vaginal tissue concentrations.[48] The work suggested that topical gel should theoretically provide greater PrEP efficacy but noted that other factors had greater influence above the antiviral effect of tenofovir.

The Centre for the AIDS Program of Research in South Africa (CAPRISA) 004 trial assessed the effectiveness and safety of a 1% vaginal gel formulation of tenofovir for the prevention of HIV acquisition in women.[33] A double-blind, randomized controlled trial was conducted comparing tenofovir gel (n = 445 women) with placebo gel (n = 444 women)

in sexually active, HIV-uninfected 18- to 40-year-old women in urban and rural KwaZulu-Natal, South Africa. The dosing strategy was based on the woman inserting a dose of gel within 12 hours before sex and a second gel dose as soon as possible and within 12 hours after sex, with a maximum two doses within 24 hours. Overall tenofovir gel reduced HIV infection by approximately 39%, with the peak effectiveness observed after 12 months of the trial at 50% protection. Participants with high (>80%), intermediate (50–80%) and low (<50%) gel adherence showed varying degrees of protection of 54%, 38% and 28% respectively. Whilst the sample size and number of sites was relatively small in this study, it provided promising evidence that coitally related dosing of tenofovir appears safe and effective in preventing HIV infection in women.

VOICE—Vaginal and Oral Interventions to Control the Epidemic was a major HIV prevention trial evaluating safety and efficacy of 3 ARV products: an oral tablet containing tenofovir, an oral tablet containing both tenofovir and emtricitabine and a tenofovir gel for vaginal administration. Despite promising results from the Partner PrEP trial, which examined both tenofovir only and tenofovir plus emtricitabine oral tablets taken once daily, the once-daily oral tenofovir only arm of VOICE was stopped early due to futility.[49] The same occurred with the vaginal gel arm, which examined the daily administration of a 1% tenofovir gel,[50] despite the CAPRISA-004 trial showing an efficacy of around 39%, where the gel was administered 12 hours before sex and a second dose as soon as possible (and within 12 hours) after sex. The result highlighted the importance of dosing regimens for the vaginal gel product.

Safety

Deeks assessed the short-term safety of tenofovir in 20 HIV-infected adults administered by intravenous infusion.[51] In this phase 1/2 clinical study, tenofovir appeared to be safe and well tolerated, with the most frequently reported adverse events mild and transient (grade I) such as headache, dizziness, fatigue and nausea. Three moderate (grade II) adverse events were also reported of nausea, fatigue and abdominal pain, all of which resolved without discontinuation of the drug. Tenofovir formulated as a once-a-day 300 mg single tablet has been extensively studied for efficacy and safety for the treatment of HIV-1 infections.[52] A 24-week investigation by Gilead into the safety of this tenofovir therapy showed a similar toxicity profile to that of placebo.[53] A larger 600-patient Gilead Sciences Study GS-99-903 (Study 903) compared a combination treatment of tenofovir, lamivudine and efavirenz with a combination treatment of stavudine, lamivudine and efavirenzin antiretroviral-naïve patients in a randomized, double-blind, parallel, placebo-controlled trial over 144 weeks.[36] During Study 903, a decrease in bone mineral density at the spine and hip was seen in the first 48 weeks but was non-progressive over the remaining weeks. The A5224s trial has also recently reported that a tenofovir-emtricitabine therapy leads to a reduction in spine and hip bone mineral density within the first 48 weeks.[54] Compared to other antiretrovirals the tenofovir-emtricitabine based therapy provided for a significant reduction in spine and hip bone mineral density. Study 903 found no significant nephrotoxicity or renal impairment, which had been reported previously among tenofovir-treated HIV patients.[55–58] An additional 336-week open-label extension phase of Study 903 for 86 patients reported no renal impairment.[59] A review by Cooper et al that assessed the findings of 17 clinical trials of tenofovir therapies, including Study 903, concluded that although the use of the drug was associated with a statistically significant loss of renal function, the clinical magnitude of this effect was modest.[60] The authors did not feel that the use of tenofovir needed to be restricted provided there was regular monitoring of renal function.

During the HPTN 050 trial of a tenofovir gel, at least one adverse event was reported by 92% of participating women; 70% of the reports involved 'reproductive system and breast disorders' (according to MedDRA coding), predominantly involving the genital tract.[47] Just under a third of the women (32%) using the gel experienced diarrhoea and general gastrointestinal symptoms. There was no specific adverse event pattern in relation to gel concentration or frequency of use. One severe adverse event occurred, a case of pelvic inflammatory disease that was possibly product related and was successfully treated with antibiotics. One moderate adverse event, shallow vulvar ulcerations, resulted in the termination of further use of the gel for that participant. The most common adverse events were genital pruritus (23%), applicator site bruising (17%), applicator site erythema (17%) and vaginal discharge (15%). There was no difference in the levels of bone fracture among participants assigned to receive tenofovir gel and the placebo gel. In CAPRISA-004, use of 1% tenofovir vaginal gel was well-tolerated.[33] While 94% of women reported at least one adverse event, there were no product-related increases in renal impairment or genital adverse events. Cases of diarrhea were reported by 17% of women, but these tended to be mild, rarely requiring medication. The vaginal gel arm of the MTN 001 trial had very limited adverse events, particularly in comparison to the oral and combination arms.[48] Transient and mild symptoms were expressed as nausea (3%) and headache (2%).

Patient Preference and Place in Therapy

The efficacy of 1% tenofovir gel is fundamentally linked to adherence, and the statistics would suggest that women are unwilling or unable to consistently use the gel as an integral part of their sex life. During the CAPRISA 004 trial, consistent (≥80%) use of the gel both before and after intercourse provided a 54% decrease in the risk of HIV infection.[33] The gel was half (28%) as effective against HIV infection if use of the gel was inconsistent (≤50%) with intercourse. Adherence promotion in the form of intensive monthly counselling and

motivational interviewing of participants only provided for overall adherence of 38% of women using the gel ≥80% before and after intercourse. The microbicide community still need to address this problem of low adherence for vaginal gels. They must clearly understand what prevents gels from being an integral part of the sex life of at-risk women; otherwise such products will have only a limited place in prophylaxis.

Is a lack of acceptability for the gel, particularly among at-risk women, the root cause of this low adherence? Women have shown a clear preference for and have a high acceptance for vaginal gels. An early study among Brazilian women examined their preferences for vaginal antimicrobial contraceptives; there was clear preference for a gel (39.6%) compared to other dosage forms, such as creams and films.[61] During the HPTN 050 trial,[62] 94% of participating women were fully adherent and said they definitely or probably would use the product if they were worried about being infected by or transmitting HIV, indicating that the gel was highly acceptable. Overall, the gel was liked by 79% of the women and 76% of male partners. However, a significant portion of these same women did report issues with the product that are already well-known disadvantages of vaginal gels. Leakage—before (41%), during (50%) and after (68%) sex—was the most common issue reported by the women. Non-intercourse-related leakage was also commonly reported. Several women found the gel messy to an unacceptable level. Some found the leakage significant, leaving them feeling moist and uncomfortable for considerable periods of time, compelling a more rigorous hygiene regime to compensate. Two thirds of women experienced leakage or messiness, while the remainder did not find the gel messy and did not experience significant leakage. Of sexually-active participants, 86% felt the gel provided for wetter sex, with a mixed reaction to whether or not this increased, decreased or made no difference to sexual pleasure. 90% of the women indicated either that the gel increased their sexual pleasure or that it made no difference.

The acceptability of the vaginal gel approach is clearly dependent on the effect the product has on the personal hygiene of the woman and on sexual intimacy with her partner. A recent review of vaginal HIV microbicides highlights clearly the importance and complexity the issue of wetness has in the acceptance of gel products in terms of personal hygiene, female preference, male preference and a women's perception of her partner's preference.[63] This review highlights the sometimes contradictory findings into the preferences for gel attributes among women and men, as well as the type of sex (dry or wet) preferred and how this influences the choices women make in continuing to use certain vaginal products. Adherence is the single most important factor controlling the effectiveness of potent microbicide gels and adherence appears tied up in the complex issue of acceptability. The 1% tenofovir gel, and other microbicidal gels that may follow, will find a place in therapy not as a single therapy in the field of vaginal microbicides but one of a number of therapies, including rings,[64,65] films,[66,67] diaphragms,[68,69] and tablets,[70] which can offer women many different choices to meet their individual needs.

Conclusion

As no HIV vaccine will likely be available in the near future, there needs to be a push towards the development of HIV microbicide products that will reduce the rate of new HIV infections. The encouraging data from the CAPRISA 004 clinical trial may do just that. However, there is an overreliance on reverse transcriptase inhibitors (RTI), like tenofovir, for use as HIV microbicides and there needs to be a move towards developing and clinically testing other potential candidates, such as entry inhibitors, integrase inhibitors and protease inhibitors, as well as various peptide- and protein-based molecules. Furthermore, the microbicide field needs to develop and clinically test a range of vaginal dosage forms, including gels, tablets, rings and films, because no one product is going to alleviate the adherence issues associated with the 1% tenofovir gel tested in the CAPRISA 004 study. A range of products needs to be made available to women, allowing them to choose the product(s) that best suits their (and their partners') sexual needs. Therefore, the 1% tenofovir gel will not be the single HIV microbicide product available, but will be part of a much wider range of products, which consists of a number of different dosage forms containing a range of different active ingredients, allowing for a dosing regimen tailored towards the individual.

Author Contributions

Conceived the concept: CM, PB, IM. Analyzed the data: CM, PB, IM. Wrote the first draft of the manuscript: CM, PB, IM. Agree with manuscript results and conclusions: CM, PB, IM. Jointly developed the structure and arguments for the paper: CM, PB, IM. Made critical revisions: CM, PB, IM. All authors reviewed and approved of the final manuscript.

DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests. Provenance: the authors were invited to submit this paper.

REFERENCES

1. HIV/AIDS: Risk behavior. *Centers for Disease Control and Prevention*. http://www.cdc.gov/hiv/risk/behavior/index.html Accessed 2nd December 2013.
2. Mann J, Tarantola D, Netter T. AIDS in the world. A global report. Part I: Chapters 2 and 3. Cambridge, MA: Harvard University Press; 1992.
3. Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science*. 1997;278 (5342):1447–1450.
4. McNeil AC, Shupert WL, Iyasere CA, et al. High-level HIV-1 viremia suppresses viral antigen-specific CD4(+) T cell proliferation. *Proc Natl Acad Sci U S A*. 2001; 98(24):13878–13883.
5. Turner BG, Summers MF. Structural biology of HIV. *Journal of Molecular Biology*. 1999;285(1):1–32.

6. Vaishnav YN, Wong-Staal F. The biochemistry of AIDS. *Annual Review of Biochemistry.* 1991;60(1):577–630.
7. Fisher AG, Feinberg MB, Josephs SF, et al. The trans-activator gene of HTLV-III is essential for virus replication. *Nature.* 1986;320:367–371.
8. Bour S, Geleziunas R, Wainberg MA. The human immunodeficiency virus type 1 (HIV-1) CD4 receptor and its central role in promotion of HIV-1 infection. *Microbiological Reviews.* 1995;59(1):63–93.
9. Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature.* 1998;393(6686):648–659.
10. Chien YW. Transdermal route of peptide and protein drug delivery. Peptide and protein drug delivery. New York: Marcel Dekker; 1992:667–689.
11. Freed EO. HIV-1 gag proteins: diverse functions in the virus life cycle. *Virology.* 1998;251(1):1–15.
12. Bounou S, Leclerc JE, Tremblay MJ. Presence of host ICAM-1 in laboratory and clinical strains of human immunodeficiency virus type 1 increases virus infectivity and CD4$^+$ T-cell depletion in human lymphoid tissue, a major site of replication in vivo. *J Virol.* 2002;76(3):1004–1014.
13. Watts C, Vickerman P. The impact of microbicides on HIV and STD transmission: model projections. *AIDS.* 2001;15:S43–S44.
14. Stone A. Microbicides: a new approach to preventing HIV and other sexually transmitted infections. *Nat Rev Drug Discov.* 2002;1(3):977–985.
15. Shattock RJ, Moore JP. Inhibiting sexual transmission of HIV-1infection. *Nat Rev Microbiol.* 2003;1(1):25–34.
16. Krebs FC, Miller SR, Catalone BJ, et al. Sodium dodecyl sulfate and C31G as microbicidal alternative 76s to nonoxynol 9: comparative sensitivity of primary human vaginal keratinocytes. *Antimicrobial Agents and Chemotherapy.* 2000;44(7):1954–1960.
17. Bestman-Smith J, Piret J, Désormeaux A, Tremblay MJ, Omar RF, Bergeron MG. Sodium lauryl sulfateabrogates human immunodeficiency virus infectivity by affecting viral attachment. *Antimicrob Agents Chemother.* 2001;45(8): 2229–2237.
18. Bax R, Douville K, McCormick D, Rosenberg M, Higgins J, Bowden M. Microbicides—evaluating multipleformulations of C31G. *Contraception.* 2002; 65(5):365–368.
19. Hillier SL. The vaginal microbial ecosystem and resistance to HIV. *AIDS Res Hum Retroviruses.* 1998;Suppl 1:S17–21.
20. Olmsted SS, Khanna KV, Ng EM, et al. Low pH immobilizes and kills human leukocytes and prevents transmission of cell-associated HIV in a mouse model. *BMC Infect Dis.* 2005;5:79.
21. Reimann KA, Khunkhun R, Lin W, Gordon W, Fung M. A humanized, nondepleting anti-CD4 antibody that blocks virus entry inhibits virus replication in rhesus monkeys chronically infected with simian immunodeficiency virus. *AIDS Res Hum Retroviruses.* 2002;18(11):747–755.
22. Vermeire K, Schols D. Cyclotriazadisulfonamides: promising new CD4-targetedanti-HIV drugs. *J Antimicrob Chemother.* 2005;56(2):270–272.
23. Van Herrewege Y, Michiels J, Van Roey J, et al. In vitro evaluation of nonnucleoside reverse transcriptase inhibitors UC-781 and TMC120-R147681 as human immunodeficiency virus microbicides. *Antimicrob Agents Chemother.* 2004;48(1):337–339.
24. Wainberg M. The prospect for RT inhibitors as topical microbicides. London: Microbicides. 2004.
25. Major I, Boyd P, Kilbourne-Brook M, Saxon G, Cohen J, Malcolm RK. A modified SILCS contraceptive diaphragm for long-term controlled release of the HIV microbicided apivirine. *Contraception.* 2013;88(1):58–66.
26. Schwebke JR. Abnormal vaginal flora as a biological risk factor for acquisition of HIV infection and sexually transmitted disease. *J Infect Dis.* 2005;192:1315–1317.
27. Rohan LC, Moncla BJ, Ayudhya RPKN, et al. In vitro and ex vivo testing of tenofovir shows it is effective as an HIV-1 microbicide. *PLoS One.* 2010; 5(2):e9310.
28. Bischofberger N, Tsai C-C, Follis KE, et al. Antiviral efficacy of PMPA in macaques chronically infected with SIV. *Antiviral Research.* 1996;30(1):A42.
29. Miller C, Rosenberg Z, Bischofberger N. Use of topical PMPA to prevent vaginal transmission of SIV. Ninth International Conference on Antiviral Research; 1996.
30. Otten RA, Smith DK, Adams DR, et al. Efficacy of postexposure prophylaxis after intravaginal exposure of pig-tailed macaques to a human-derived retrovirus (human immunodeficiency virus type 2). *Journal of Virology.* 2000; 74(20):9771–9775.
31. Tsai CC, Follis KE, Sabo A, et al. Prevention of SIV infection in macaques by (R)-9-(2-phosphonylmethoxypropyl) adenine. *Science.* 1995;270(5239):1197–1199.
32. Parikh UM, Dobard C, Sharma S, et al. Complete protection from repeated vaginal simian-human immunodeficiency virus exposures in macaques by a topical gel containing tenofovir alone or with emtricitabine. *Journal of Virology.* 2009;83(20):10358–10365.
33. Karim QA, Karim SSA, Frohlich JA, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science.* 2010;329(5996):1168–1174.
34. Tan DH, Kaul R, Raboud JM, Walmsley SL. No impact of oral tenofovir disoproxilfumarate on herpes simplex virus shedding in HIV-infected adults. *AIDS.* 2011;25(2):207–210.
35. Andrei G, Lisco A, Vanpouille C, et al. Topical tenofovir, a microbicide effective against HIV, inhibits herpes simplex virus-2 replication. *Cell Host & Microbe.* 2011;10(4):379–389.
36. Gallant JE, Staszewski S, Pozniak AL, et al. Efficacy and safety of tenofovir DF vs. stavudine in combination therapy in antiretroviral-naive patients. *Journal of the American Medical Association.* 2004;292(2):191–201.
37. Gallant JE, Gallant JE, Rodriguez AE, et al. Early virologic nonresponse to tenofovir, abacavir, and labivudine in HIV-Infected antiretroviral-naive subjects. *Journal of Infectious Diseases.* 2005;192(11):1921–1930.
38. Molina JM, Andrade-Villanueva J, Echevarria J, et al. Once-daily atazanavir/ritonavir versus twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, for management of antiretroviral-naive HIV-1-infected patients: 48 week efficacy and safety results of the CASTLE study. *Lancet.* 2008;372(9639): 646–655.
39. Martin A, Bloch M, Amin J, et al. Simplification of Antiretroviral Therapy with Tenofovir-Emtricitabine or Abacavir-Lamivudine A Randomized, 96-Week Trial. *Clinical Infectious Diseases.* 2009;49(10):1591–1601.
40. Molina JM, Cahn P, Grinsztejn B, et al. Rilpivirine versus efavirenz with tenofovir and emtricitabine in treatment-naive adults infected with HIV-1 (ECHO): a phase 3 randomised double-blind active-controlled trial. *Lancet.* 2011;378(9787):238–246.
41. Sax PE, DeJesus E, Mills A, et al. Co-formulated elvitegravir, cobicistat, emtricitabine, and tenofovir versus co-formulated efavirenz, emtricitabine, and tenofovir for initial treatment of HIV-1 infection: a randomised, double-blind, phase 3 trial, analysis of results after 48 weeks. *Lancet.* 2012;379(9835):2439–2448.
42. Chi BH, Sinkala M, Mbewe F, et al. Single-dose tenofovir and emtricitabine for reduction of viral resistance to non-nucleoside reverse transcriptase inhibitor drugs in women given intrapartum nevirapine for perinatal HIV prevention: an open-label randomised trial. *Lancet.* 2007;370(9600):1698–1705.
43. Taburet AM, Piketty C, Chazallon C, et al. Interactions between atazanavir-ritonavir and tenofovir in heavily pretreated human immunodeficiency virus-infected patients. *Antimicrobial Agents and Chemotherapy.* 2004;48(6):2091–2096.
44. Van Damme L, Corneli A, Ahmed K, et al. Preexposure prophylaxis for HIV infection among African women. *New England Journal of Medicine.* 2012;367(5): 411–422.
45. Thigpen MC, Kebaabetswe PM, Paxton LA, et al. Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. *New England Journal of Medicine.* 2012;367(5):423–434.
46. Baeten JM, Donnell D, Ndase P, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *New England Journal of Medicine.* 2012;367(5):399–410.
47. Mayer KH, Maslankowski LA, Gai F, et al. Safety and tolerability of tenofovir vaginal gel in abstinent and sexually active HIV-infected and uninfected women. *AIDS.* 2006;20(4):543–551.
48. Hendrix CW, Chen BA, Guddera V, et al. MTN-001: randomized pharmacokinetic cross-over study comparing tenofovir vaginal gel and oral tablets in vaginal tissue and other compartments. *PloS One.* 2013;8(1):e55013.
49 NIH modifies 'VOICE' HIV prevention study in women: oral tenofovir discontinued in clinical trial. Bethesda, MD: National Institute of Allergy and Infectious Diseases, September 28, 2011. (http://www.nih.gov/news/health/sep2011/niaid-28.htm)
50. MTN statement on decision to discontinue use of tenofovir gel in VOICE, major HIV prevention study in women. Pittsburgh: Microbicide Trials Network, November 25, 2011 (http://www.mtnstopshiv.org/node/3909)
51. Deeks SG, Barditch-Crovo P, Lietman PS, et al. Safety, pharmacokinetics, and antiretroviral activity of intravenous 9-[2-(R)-(phosphonomethoxy) propyl] adenine, a novel anti-human immunodeficiency virus (HIV) therapy, in HIV-infected adults. *Antimicrobial Agents and Chemotherapy.* 1998;42(9): 2380–2384.
52. Fung HB, Stone EA, Piacenti FJ. Tenofovirdisoproxilfumarate: a nucleotide reverse transcriptase inhibitor for the treatment of HIV infection. *Clinical Therapeutics.* 2002;24(10):1515–1548.
53. Cheng A, Barriere S, Coakley D, Chen S, Wulfsohn M, Toole J. Safety profile of tenofovir DF (TDF) in treatment-experienced patients from randomized, placebo-controlled clinical trials. 14th International AIDS Conference; 2002.
54. McComsey GA, Kitch D, Daar ES, et al. Bone mineral density and fractures in antiretroviral-naive persons randomized to receive abacavir-lamivudine or tenofovirdisoproxilfumarate-emtricitabine along with efavirenz or atazanavir-ritonavir: Aids Clinical Trials Group A5224s, a substudy of ACTG A5202. *Journal of Infectious Diseases.* 2011;203(12):1791–1801.
55. Karras A, Lafaurie M, Furco A, et al. Tenofovir-related nephrotoxicity in human immunodeficiency virus-infected patients: three cases of renal failure, Fanconi syndrome, and nephrogenic diabetes insipidus. *Clinical Infectious Diseases.* 2003; 36(8):1070–1073.

56. Créput C, Gonzalez-Canali G, Hill G, Piketty C, Kazatchkine M, Nochy D. Renal lesions in HIV-1-positive patient treated with tenofovir. *AIDS*. 2003; 17(6):935–937.
57. Rollot F, Nazal E-M, Chauvelot-Moachon L, et al. Tenofovir-related Fanconi syndrome with nephrogenic diabetes insipidus in a patient with acquired immunodeficiency syndrome: the role of lopinavir-ritonavir-didanosine. *Clinical Infectious Diseases*. 2003;37(12):e174–e6.
58. Schaaf B, Aries S, Kramme E, Steinhoff J, Dalhoff K. Acute renal failure associated with tenofovir treatment in a patient with acquired immunodeficiency syndrome. *Clinical Infectious Diseases*. 2003;37(3):e41–e3.
59. Izzedine H, Hulot JS, Vittecoq D, et al. Long-term renal safety of tenofovirdisoproxilfumarate in antiretroviral-naive HIV-1-infected patients. Data from a double-blind randomized active-controlled multicentre study. *Nephrology Dialysis Transplantation*. 2005;20(4):743–746.
60. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic review and meta-analysis: renal safety of tenofovirdisoproxilfumarate in HIV-infected patients. *Clinical Infectious Diseases*. 2010;51(5):496–505.
61. Hardy E, Jiménez AL, de Pádua KS, Zaneveld LJ. Women's preferences for vaginal antimicrobial contraceptives III: Choice of a formulation, applicator, and packaging. *Contraception*. 1998;58(4):245–249.
62. Rosen RK, Morrow KM, Carballo-Diéguez A, et al. Acceptability of tenofovir gel as a vaginal microbicide among women in a phase I trial: a mixed-methods study. *Journal of Women's Health*. 2008;17(3):383–392.
63. Domanska CA, Teitelman AM. Factors that affect acceptance of HIV microbicides among women. *Collegian: Journal of the Royal College of Nursing Australia*. 2012;19(1):23–32.
64. Nel A, Smythe S, Young K, et al. Safety and pharmacokinetics of dapivirine delivery from matrix and reservoir intravaginal rings to HIV-negative women. *Journal of Acquired Immune Deficiency Syndromes*. 2009;51(4):416–423.
65. Smith JM, Rastogi R, Teller RS, et al. Intravaginal ring eluting tenofovirdisoproxilfumarate completely protects macaques from multiple vaginal simian-HIV challenges. Proceedings of the National Academy of Sciences. 2013;110 (40):16145–50.
66. Ham AS, Rohan LC, Boczar A, Yang L, Buckheit KW, Buckheit Jr RW. Vaginal film drug delivery of the pyrimidinedione IQP-0528 for the prevention of HIV infection. *Pharmaceutical Research*. 2012;29(7):1897–1907.
67. Patton D, Sweeney YC, Rohan L, Hillier S. P3. 365 tenofovir vaginal film: safety assessment in the macaque model. *Sexually Transmitted Infections*. 2013;89 (Suppl 1):A263–A.
68. Major I, Boyd P, Kilbourne-Brook M, Saxon G, Cohen J, Malcolm RK. A modified SILCS contraceptive diaphragm for long-term controlled release of the HIV microbicide dapivirine. *Contraception*. 2012;88(1):58–66.
69. Frezieres RG, Walsh T, Kilbourne-Brook M, Coffey PS. Couples' acceptability of the SILCS diaphragm for microbicide delivery. *Contraception*. 2012;85(1):99–107.
70. McConville C, Friend DR, Clark MR, Malcolm K. Preformulation and development of a once-daily sustained-release tenofovir vaginal tablet tablet containing a single excipient. *J Pharm Sci*. 2013;102(6):1859–1868.